

Preliminary Amendment

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Applicant(s): Stanton et al.

Serial No.: 09/641,801

Confirmation No.: 5388

Filed: August 17, 2000

For: USE OF COLOSTRININ, CONSTITUENT PEPTIDES THEREOF, AND ANALOGS THEREOF FOR INDUCING CYTOKINES

peptides are described in International Patent Publication No. WO 00/75173, filed June 2, 2000, claiming priority to June 2, 1999, and can be synthesized according to the general method described in the Examples Section. These peptides (i.e., constituent peptides of colostrinin), which can be derived from colostrinin or chemically synthesized, include: MQPPPLP (SEQ ID NO:1); LQTPQPLLQVMMEPQGD (SEQ ID NO:2); DQPPDVEKPDLQPFQVQS (SEQ ID NO:3); LFFFLPVNVLP (SEQ ID NO:4); DLEMPVLPVEPFPPV (SEQ ID NO:5); MPQNFYKLPQM (SEQ ID NO:6); VLEMKFPPPPQETVT (SEQ ID NO:7); LKPFPKLKVEVFPFP (SEQ ID NO:8); VVMEV (SEQ ID NO:9); SEQP (SEQ ID NO:10); DKE (SEQ ID NO:11); FPPPK (SEQ ID NO:12); DSQPPV (SEQ ID NO:13); DPPPPQS (SEQ ID NO:14); SEEMP (SEQ ID NO:15); KYKLQPE (SEQ ID NO:16); VLPPNVG (SEQ ID NO:17); VYPFTGPIPN (SEQ ID NO:18); SLPQNILPL (SEQ ID NO:19); TQTPVVVPPF (SEQ ID NO:20); LQPEIMGVPKVKETMVPK (SEQ ID NO:21); HKEMPFPKYPVEPFTESQ (SEQ ID NO:22); SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23); SWMHQPP (SEQ ID NO:24); QPLPPTVMFP (SEQ ID NO:25); PQSVLS (SEQ ID NO:26); LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27); AFLLYQE (SEQ ID NO:28); RGPFPILV (SEQ ID NO:29); ATFNRYQDDHGEEILKSL (SEQ ID NO:30); VESYVPLFP (SEQ ID NO:31); FLLYQEPVLGPVR (SEQ ID NO:32); LNF (SEQ ID NO:33); and MHQPPQPLPPTVMFP (SEQ ID NO:34). These can be classified as follows: (A) those of unknown precursor include SEQ ID NOs:2, 6, 7, 8, 10, 11, 14, and 33; (B) those having a β -casein homologue precursor include SEQ ID NOs:1, 3, 4, 5, 9, 12, 13, 15, 16, 17, and 31; (C) those having a β -casein precursor include SEQ ID NOs:18 (casein amino acids 74-83), 19 (casein amino acids 84-92), 20 (casein amino acids 93-102), 21 (casein amino acids 103-120), 22 (casein amino acids 121-138), 23 (casein amino acids 139-156), 24 (casein amino acids 157-163), 25 (casein amino acids 164-173), 26 (casein amino

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acids 174-179), 27 (casein amino acids 180-201), 28 (casein amino acids 202-208), 29 (casein amino acids 214-222), 32 (casein amino acids 203-214), and 34 (casein amino acids 159-173); and (D) those having an annexin precursor include SEQ ID NO:30 (annexin amino acids 203-220).

Please replace the paragraph at page 20, line 38 to line 41, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A3, with notations to indicate the changes made.

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Cytokine studies: Colostrinin has previously been shown in the literature to induce IFN- γ and TNF- α , as has Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro (SEQ ID NO:31), which is disclosed in International Publication No. WO-A-98/14473. Thus, studies were done to investigate the individual peptides.

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Please replace the paragraph at page 26, line 3 to page 29, line 16, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A4, with notations to indicate the changes made.

Table 2. (cont.) Cytokines induced in human leukocyte cultures stimulated with CCP, colostrum or commercial milk formulas.

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PEPTIDE (Exp. #)	PEPTIDE CONCENTRATION (pg/ml) (mg/ml)	IL-4	IL-6 (pg/ml)	IL-12 (pg/ml)
Example 1				
SEQ ID NO:1	100	0	235.4	0
	10	0	934.8	0
	1	0	675.3	0
	0.1	0	497.1	0
SEQ ID NO:7	100	0	291.3	0
	10	0	645.4	0
SEQ ID NO:8	100	0	1076	0
	10	0	1024	0
	1	0	1013	0
	0.1	0	533.6	0
SEQ ID NO:3	1	0	620.5	0
	0.1	0	107	0
SEQ ID NO:2	100	0	258.6	0
	10	0	551.3	0
	1	0	1205	0
	0.1	0	325	0

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SEQ ID NO:4	10	0	1718	0
	1	0	744.4	0
SEQ ID NO:5	100	0	98.2	0
	10	0	750	0
SEQ ID NO:6	100	0	63.3	0
	10	0	864.5	0
SEQ ID NO:31	100	1.4	1489	0
	10	0	836.3	0
	1	0.4	489.9	0
	0.1	2.4	1635	0
Colostrinin	10	0	1832	0
	1	1.9	1915	0
	0.1	0.4	430.1	0
Raw Colostrum	100	0	1879	0
	10	0	602.2	0
	1	0	1055	0
	0.1	5.0	187.2	0
Control		0	13.5	0
SEA		4	1704	0

Example 2

SEQ ID NO:18	100	0		0
SEQ ID NO:19	10	0		0
	1	33.8		0
SEQ ID NO:20	100	0		0

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	10	0.4		0
SEQ ID NO:22	100	41.5		0
	10	32.7		0
	1	30.1		0
	0.1	17.8		0
SEQ ID NO:1	100	0		0
	10	3.5		5.7
	1	26.6		0
	0.1	47.6		0
SEQ ID NO:7	100	24.5		0
SEQ ID NO:2	100	22.5		33.5
	10	19.9		0
	1	10.1		9.9
	0.1	29.1		2.2
Enfamil	1:5	0.9		0
Low Iron	1:10	4.0		0
Enfamil	1:5	0		0
with Iron	1:10	0		0
Control		0		0
SEA		62.5		54.8

Example 3

SEQ ID NO:1	100	0	942.5	0
	10	ND	ND	ND
SEQ ID NO:7	1	0	32.9	0
	0.1	ND	ND	ND

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SEQ ID NO:8	10	0	18.5	4.0
	1	ND	ND	ND
SEQ ID NO:5	100	0	0	0
Raw	100	0	0	0
Colostrum	10	0	1853	1.6
	1	ND	ND	ND
	0.1	ND	ND	ND
Colostrinin	10	0	2009	17.6
	1	0	1861	7.5
	0.1	ND	ND	ND
SEQ ID NO:31	10	0	16.8	18.7
	1	0	722.9	0
	0.1	ND	ND	ND
SEQ ID NO:22	100	6.0	1630	0
	10	0	46.7	0
	1	0	0	0
	0.1	ND	ND	ND
Enfamil Low Iron	1:5	0	1913	0
Enfamil with Iron	1:5	0.4	1953	0
Control		0	0	0
SEA		16.8	866.2	0

*SEQ ID NOs:1-8 and 31, Raw Colostrum, and Colostrinin were reconstituted on the same day.

*SEQ ID NOs:18, 19, 20, and 22 were reconstituted on the same day.

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Please replace the paragraph at page 30, line 1 to page 31, line 24, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A, with notations to indicate the changes made.

Table 3. Relative abilities of the various peptides to induce cytokines and proliferation

	Ex. 1	Ex. 2	Ex. 1	Ex. 1	Ex. 1	Ex. 2	Ex. 1
Rank	IFN- γ	IFN- γ	Micro. Resp.	Prolif. Resp.	TNF- α	TNF- α	IL-10
1	SEQ ID NO:8	SEQ ID NO:1	SEQ ID NO:8	SEQ ID NO:2	SEQ ID NO:2**	SEQ ID NO:2	SEQ ID NO:8
2	SEQ ID NO:31	SEQ ID NO:2	SEQ ID NO:2	SEQ ID NO:1	SEQ ID NO:8	SEQ ID NO:1	SEQ ID NO:1
3	SEQ ID NO:2	SEQ ID NO:7	SEQ ID NO:31	SEQ ID NO:4	SEQ ID NO:31	SEQ ID NO:7	SEQ ID NO:3
4	SEQ ID NO:1	SEQ ID NO:22	SEQ ID NO:1	Colostrum	Colostrum	SEQ ID NO:22	SEQ ID NO:2
5	SEQ ID NO:3	SEQ ID NO:19	SEQ ID NO:7	Colostrinin	Colostrinin	SEQ ID NO:19	Colostrinin
6	Colistrinin	SEQ ID NO:20	Colostrinin	SEQ ID NO:8	SEQ ID NO:3	SEQ ID NO:20	Colostrum
7	Colustrum	SEQ ID NO:18	Colostrum	SEQ ID NO:31	SEQ ID NO:1	SEQ ID NO:18	SEQ ID NO:31
8	SEQ ID NO:4		SEQ ID NO:3	SEQ ID NO:5	SEQ ID NO:5		SEQ ID NO:4

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9	SEQ ID NO:5	SEQ ID NO:4	SEQ ID NO:6	SEQ ID NO:7	Low Enfamil	SEQ ID NO:7
9	SEQ ID NO:6	SEQ ID NO:5	SEQ ID NO:7	SEQ ID NO:4	High Enfamil	SEQ ID NO:5
10	SEQ ID NO:7	SEQ ID NO:6	SEQ ID NO:3	SEQ ID NO:6		SEQ ID NO:6

* SEQ ID NO:7 < 2 fold difference in titer

** All good inducers

*** No difference in titer

Table 3. (Cont.) Relative abilities of the various peptides to induce cytokines and proliferation

Rank	Ex. 2 IL-10	Ex. 1 IL-4	Ex. 2 IL-4	Ex. 1 IL-6	Ex. 1 IL-12	Ex. 2 IL-12
1	SEQ ID NO:2	Colostrum	SEQ ID NO:1	SEQ ID NO:31	All neg.	SEQ ID NO:2
2	SEQ ID NO:7	Colostrinin	SEQ ID NO:2	SEQ ID NO:8		SEQ ID NO:1
3	SEQ ID NO:1	SEQ ID NO:31	SEQ ID NO:22	SEQ ID NO:1		
4	SEQ ID NO:19		SEQ ID NO:19	Colostrinin		
5	SEQ ID NO:22		SEQ ID NO:7	SEQ ID NO:2		

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